FOODBORNE PATHOGENS AND DISEASE
Volume 6, Number 7, 2009

DOI: 10.1089/fpd.2009.0306

Review

Dietary Interactions and Interventions Affecting *Escherichia coli* O157 Colonization and Shedding in Cattle*

Megan E. Jacob,[1] Todd R. Callaway,[2] and T.G. Nagaraja[1]

Abstract

Escherichia coli O157 is an important foodborne pathogen affecting human health and the beef cattle industry. Contamination of carcasses at slaughter is correlated to the prevalence of *E. coli* O157 in cattle feces. Many associations have been made between dietary factors and *E. coli* O157 prevalence in cattle feces. Preharvest interventions, such as diet management, could reduce the fecal prevalence and diminish the impact of this adulterant. Dietary influences, including grain type and processing method, forage quality, and distillers grains have all been associated with *E. coli* O157 prevalence. In addition, several plant compounds, including phenolic acids and essential oils, have been proposed as in-feed intervention strategies. The specific mechanisms responsible for increased or decreased *E. coli* O157 shedding or survival are not known but are often attributed to changes in hindgut ecology induced by diet types. Some interventions may have a direct bacterial effect. Frequently, results of studies are conflicting or not repeatable, which speaks to the complexity of the hindgut ecosystem, variation in animal feed utilization, and variation within feed products. Still, understanding specific mechanisms, driven by diet influences, responsible for *E. coli* O157 shedding will aid in the development and implementation of better and practical preharvest intervention strategies.

Introduction

According to the U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) estimates, more than 33 million pounds of beef products were recalled during 2007, and more than 7 million pounds were recalled in 2008 for possible *Escherichia coli* O157:H7 contamination (http://www.fsis.usda.gov/FSIS_Recalls/). These dramatic numbers indicate potential health implications for humans and economic repercussions for the beef industry. *E. coli* O157, a Shiga toxin–producing serotype of *E. coli*, is an important foodborne pathogen associated with enteritis in thousands of people in the United States every year. In more severe cases, infection can lead to hemolytic uremic syndrome, and possibly death (Rangel *et al.*, 2005). In cattle, a primary reservoir of *E. coli* O157, the organism colonizes the gut and is shed in the feces. Cattle feces are a major source of contamination of food products (Rangel *et al.*, 2005). Because of the risk to human health and economic burden of recalls to the cattle industry, it is important to understand the ecology of *E. coli* O157 in cattle, as well as develop and implement strategies to reduce colonization and shedding.

E. coli O157 generally colonizes the lower gastrointestinal tract of cattle, specifically the mucosal area of the terminal rectum (Naylor *et al.*, 2003; Low *et al.*, 2005). Calves are likely colonized early in life (Gannon *et al.*, 2002), and the organism is generally considered part of the normal gastrointestinal flora of cattle and not associated with disease. Presence of *E. coli* O157 in cattle feedlots appears fairly ubiquitous (Sargeant *et al.*, 2003); however, several variables, including season, geographic location, and diet, have been associated with increased prevalence (Herriott *et al.*, 1998; Bach *et al.*, 2002; Dewell *et al.*, 2005; Chase-Topping *et al.*, 2008; Renter *et al.*, 2008). Although many associations have been made, reasons why specific factors influence *E. coli* O157 presence in cattle remain largely unknown.

Because of its location in the gut, *E. coli* O157 colonization and survival are likely affected by gastrointestinal conditions, including pH, concentration of volatile fatty acids (VFA), presence of competing organisms, and, possibly, other unknown factors. Diet, which impacts the gastrointestinal conditions, is frequently associated with *E. coli* O157 prevalence (Dargatz *et al.*, 1997; Herriott *et al.*, 1998; Callaway *et al.*, 2003b; Fox *et al.*, 2007; Jacob *et al.*, 2008a). Often, results of studies

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, Manhattan, Kansas.
[2]Food and Feed Safety Research Unit, Southern Plains Agricultural Research Center, Agricultural Research Service, U.S. Department of Agriculture, College Station, Texas.
*Contribution no. 09-261-J from the Kansas Agricultural Experiment Station.

evaluating associations between diets or diet components and *E. coli* O157 prevalence are conflicting or not repeatable, which speaks to the complexity of hindgut ecology and mechanisms responsible for increased or decreased colonization and fecal shedding. Still, feeds and feeding management have been proposed as possible preharvest intervention strategies (Callaway *et al.*, 2003a; Loneragan and Brashears, 2005; LeJeune and Wetzel, 2007). The primary objective of this review is to highlight dietary components or practices that have been associated with *E. coli* O157 prevalence in ruminants, primarily cattle, and provide insight into factors that may be beneficial in developing intervention strategies. Although feed additives currently in use or being developed, including ionophores, probiotics, and sodium chlorate, have been associated with *E. coli* O157 prevalence, they are not included in this review.

Grain Type and Processing

Cattle are typically fed high-energy grain diets to increase weight gain and efficiency of feed conversion; grain type in diets has been linked to *E. coli* O157 prevalence. Barley grain has been positively associated with *E. coli* O157 shedding in both observational and experimental studies (Dargatz *et al.*, 1997; Buchko *et al.*, 2000; Berg *et al.*, 2004). Specifically, Berg *et al.* (2004) reported that cattle shed a higher concentration of *E. coli* O157 and had higher fecal pH when fed a barley grain diet compared with cattle fed a corn-based diet. Interestingly, concentrations of generic *E. coli* populations were higher in corn-fed cattle (6.2 log CFU/g) than in barley-fed cattle (5.6 log CFU/g), similar to the grain and forage effect reported by Diez-Gonzalez *et al.* (1998). Although hypothesized to be a change in hindgut ecology, the specific mechanism responsible for increased *E. coli* O157 shedding in barley-fed cattle is not known. Barley, which has lower starch content than other traditional cereal grains (Huntington, 1997), is more rapidly and completely digested in the rumen (Ørskov, 1986; Theurer, 1986) and results in less undigested starch for secondary fermentation in the large intestine. Therefore, cattle fed barley grain–based diets have an increased pH and decreased VFA concentrations in the hindgut. A study to evaluate the survival of inoculated *E. coli* O157 in fecal samples from cattle fed either barley or corn diets found few differences in the pathogen survival; however, pH and VFA concentrations were generally similar between the two diets before *E. coli* O157 disappeared (Bach *et al.*, 2005b).

In addition to the hypothesized rationale of increased hindgut starch concentration in corn-fed cattle, one study evaluated effects of supplementing canola oil in barley- and corn-based diets because the total oil content between the two grain types is likely different and may impact hindgut conditions (Bach *et al.*, 2005a). Fats or oils could have a direct impact on *E. coli* O157 because fatty acids, particularly unsaturated fatty acids, have antibacterial activity (Galbraith and Miller, 1973). Fecal shedding of inoculated *E. coli* O157 was not different between diets in this study, and although fecal pH was lower and VFA concentrations were higher in corn-fed than in barley-fed cattle, there was no effect on shedding, fecal pH, or VFA concentration with supplementation of canola oil (Bach *et al.*, 2005a).

An association between *E. coli* O157 prevalence and diets containing cottonseed has also been inconsistent. Cottonseed, which has high oil content, could affect the hindgut ecosystem. Garber *et al.* (1995) reported a negative association between feeding whole cottonseed to heifers and fecal shedding of *E. coli* O157 in a case–control study; Hancock *et al.* (1994) reported similar findings. Others have shown no relationship between the two factors (Dargatz *et al.*, 1997; Herriott *et al.*, 1998; Buchko *et al.*, 2000). Several other positive associations between feed types like corn silage (Herriott *et al.*, 1998) and *E. coli* O157 shedding in cattle have been reported sporadically, but again, these observations are limited (e.g., Dargatz *et al.*, 1997).

The processing method used to prepare cereal grains for cattle diets also affects substrate availability in the hindgut. Processing grains with heat, moisture, or mechanical treatment will increase starch degradation in the rumen, which in turn influences starch availability and fermentation in the lower gastrointestinal tract (Huntington, 1997). Fox *et al.* (2007) reported that grain-processing method affected *E. coli* O157 prevalence in cattle. In that study, heifers fed steam-flaked grains (more completely digested in the rumen) had higher *E. coli* O157 prevalence than heifers fed dry-rolled grain diets (less completely digested in the rumen) on most sampling days. They hypothesized that dry-rolled grains provided more substrate (starch) to the hindgut, reducing pH and creating an inhospitable environment for *E. coli* O157. The authors measured fecal pH as an indicator for fermentation activity in the hindgut and found no significant difference between cattle fed the two types of processed grains (Fox *et al.*, 2007). Depenbusch *et al.* (2008) also showed a trend of higher *E. coli* O157 prevalence in cattle fed steam-flaked grain diets compared with cattle fed dry-rolled grain diets for 30 days. In one of the two experiments, positive *E. coli* O157 samples were associated with greater fecal starch concentration; however, neither fecal starch nor fecal pH was associated with *E. coli* O157–positive samples in a second experiment (Depenbusch *et al.*, 2008). An increased fecal starch concentration does not support the hypothesis that increased substrate negatively affects *E. coli* O157. In an observational study of cattle in Midwestern feedlots, Dewell *et al.* (2005) found no significant effect of grain processing on *E. coli* O157 prevalence in cattle.

In an Australian study, steam-flaked sorghum or rolled barley resulted in increased fecal generic *E. coli* concentrations compared with diets with whole sorghum or barley (Gilbert *et al.*, 2005). The whole grains were associated with higher fecal starch concentrations and higher fecal pH. Because fecal pH and starch concentration are not consistently associated with *E. coli* O157 (Gilbert *et al.*, 2005; Depenbusch *et al.*, 2008), neither is likely entirely responsible for the association of steam-flaked grains with higher *E. coli* O157 prevalence. Still, it does appear that different processing methods (steam-flaking and dry-rolling) may affect *E. coli* prevalence and concentrations.

Forage Quality

Similar to the difference reported between grain type and processing, differences in *E. coli* O157 prevalence have been reported in ruminants fed diets with different forage qualities. Kudva *et al.* (1995) reported that switching experimentally inoculated sheep from an alfalfa pellet diet to a low-quality forage diets increased *E. coli* O157 shedding. In another study, *E. coli* O157 was inoculated in fecal samples from cattle fed straw, low-digestible grass silage, and highly digestible grass silage plus maize silage, and the survival was analyzed (Franz *et al.*, 2005). The authors reported a faster rate of decline in concentrations of *E. coli* O157 in low-quality forages, associated with higher pH and fiber content, which contradicts the work of Kudva *et al.* (1995). Perhaps these differences could be explained by phenolic acids found in the different forage types (described later).

Forage and Grain Diets

Studies evaluating effects of forage and grain diets on the fecal shedding of *E. coli* O157 in ruminants are perhaps the most numerous and conflicting (Table 1). Experimental inoculation studies of both sheep and cattle have shown that animals fed forage diets shed *E. coli* O157 in the feces for a longer duration than animals consuming grain-based diets (Kudva *et al.*, 1997; Hovde *et al.*, 1999; Van Baale *et al.*, 2004). However, not all studies have found significant differences in *E. coli* O157 prevalence between these feed types (Tkalcic *et al.*, 2000; Fegan *et al.*, 2004). Additionally, a study evaluating survival of inoculated *E. coli* O157 in manure samples from cattle fed hay- or silage-based diets had conflicting results, which were related to the duration the donor animals were on feed (Wells *et al.*, 2005).

Generally, the rationale for a positive association is an increased ruminal and/or hindgut pH and decreased VFA concentrations associated with the forage diet, which contribute to a more hospitable environment for *E. coli* O157 survival and colonization. Van Baale *et al.* (2004) found an increased fecal and ruminal pH in calves fed forage diets, which complemented their *E. coli* O157 shedding results. This rationale is logical considering that when ruminants are fed grain diets, starch can be fermented in the rumen or pass through before secondary fermentation occurs in the cecum and colon, lowering pH and increasing VFA concentrations (Huntington, 1997). Russell *et al.* (2000) reported that grain feeding had a greater effect on fermentation and bacterial populations in the hindgut than in the rumen.

Interestingly, Diez-Gonzalez *et al.* (1998) reported significantly higher total *E. coli* concentrations in feces of cattle fed concentrate diets compared with feces from cattle fed forage diets. As expected, lower pH and higher VFA concentrations were observed in cattle fed grain than in cattle fed forage diets (Diez-Gonzalez *et al.*, 1998). Similar findings with generic *E. coli* have been reported by others (Krause *et al.*, 2003; Gilbert *et al.*, 2005). The association between these generic *E. coli* and *E. coli* O157 populations is not known. However, Diez-Gonzalez *et al.* (1998) reported that increased concentrations of acid-resistant *E. coli* were observed in cattle fed diets with grain than in cattle fed a diet with no grain. It has been suggested that *E. coli* O157 survival is favored in low pH and high VFA concentration conditions (Russell *et al.*, 2000). Acid resistance has been shown to occur in *E. coli* O157 incubated in

Table 1. Effects of Feeding Grain- or Forage-Based Diets on *Escherichia coli* O157 and Generic *E. coli* Shedding or Survival in Ruminant Feces

Diet	*Organism*	*Study design*	*Results*	*References*
100% grass vs. 50% corn and 50% alfalfa	*E. coli* O157	Sheep: experimental inoculation	Increased length of shedding in forage-fed	Kudva *et al.*, 1997
62% barley + 19% corn, 90% corn, 100% alfalfa, and 100% hay	*E. coli* O157	Cattle: experimental inoculation	Increased length of shedding in hay-fed	Hovde *et al.*, 1999
1.9 kg Bermuda grass + 3.8 kg concentrate mix vs. 3.8 kg Bermuda grass + 1.9 kg concentrate mix	*E. coli* O157	Cattle: experimental inoculation	No difference in fecal shedding	Tkalcic *et al.*, 2000
90% grain + 10% silage vs. 50% alfalfa hay + 50% grass hay	*E. coli* O157	Cattle: experimental inoculation	No difference in fecal shedding	Grauke *et al.*, 2003
Grass-fed vs. lot-fed	*E. coli* O157	Cattle: observation of natural prevalence	No difference in prevalence or concentration	Fegan *et al.*, 2004
85% forage + 15% grain vs. 15% forage + 85% grain	*E. coli* O157	Cattle: experimental inoculation	Increased length of shedding in forage-fed	Van Baale *et al.*, 2004
100% hay vs. 88% corn silage + 9% cracked corn	*E. coli* O157	Cattle: inoculation of feces	Death rate dependent on animal time on feed	Wells *et al.*, 2005
No grain, 60% rolled corn, 80% rolled corn	Generic *E. coli*	Cattle: observation of natural *E. coli* population	Higher concentration in grain-fed	Diez-Gonzalez *et al.*, 1998
100% forage (grass) vs. 70% rolled sorghum +30% grass	Generic *E. coli*	Cattle: observation of natural *E. coli* population	Higher concentration in grain-fed	Krause *et al.*, 2003
Roughage (±50% molasses) vs. 80% grain	Generic *E. coli*	Cattle: observation of natural *E. coli* population	Higher concentration in grain-fed	Gilbert *et al.*, 2005

rumen fluid (Tkalcic *et al.*, 2000). However, not all studies report differences in acid-resistance of *E. coli* O157 between grain- and forage-based diets (Hovde *et al.*, 1999; Van Kessel *et al.*, 2002; Grauke *et al.*, 2003). Fu *et al.* (2003) speculated that *E. coli* O157 growth and acid resistance depend on both pH and VFA concentrations. Others have shown that under anaerobic conditions, short-chained fatty acids in human fecal samples can suppress *E. coli* O157 growth (Shin *et al.*, 2002). More complete reviews on acid resistance and other forage feeding effects have been published previously (Russell *et al.*, 2000; Callaway *et al.*, 2003b). Nevertheless, the role of acid resistance on *E. coli* O157 survival and prevalence is highly debated and still not well understood.

Distillers Grains

Because of increased availability due to increased ethanol production, distillers grains, an ethanol fermentation coproduct usually derived from corn, are included in cattle diets as a protein and energy source (Klopfenstein *et al.*, 2008). After the starch from corn is fermented to ethanol, the remaining nutrients (protein, fiber, and fat) are concentrated approximately threefold and fed to cattle in a wet or dehydrated form (Klopfenstein *et al.*, 2008). Other cereal grains can be fermented in a similar manner. Several studies have demonstrated an association between feeding ethanol coproducts (distillers or brewers grains) and *E. coli* O157 prevalence in cattle. In 2003, Synge *et al.*, investigating management factors associated with *E. coli* O157 shedding, initially reported an association in Scottish cattle fed distillers grains. This observation was also seen in U.S. feedlots with brewers grains, a coproduct of the brewing industry (Dewell *et al.*, 2005). Differences in the probability of detecting *E. coli* O157 in the terminal rectum of cattle fed varying levels of distillers grains were reported in a vaccine trial; however, the relationship was not linear (Peterson *et al.*, 2007). In a study aimed at evaluating the effect of feeding distillers grains on *E. coli* O157 shedding, cattle fed dried distillers grains with solubles (DDGS) at 25% of the final diet had a twofold higher prevalence of the organism than cattle not fed DDGS (Jacob *et al.*, 2008a). Likewise, a challenge model using calves orally inoculated with *E. coli* O157 and fed one of two diets, with or without 25% DDGS, found that calves fed distillers grains shed higher concentrations of *E. coli* O157 at the end of the study and had a higher concentration in gut contents at necropsy than calves in the control group (Jacob *et al.*, 2008b). Persistence of the organism was also different in experimentally inoculated manure slurries from cattle fed varying levels of wet distillers grains with solubles (WDGS) (Varel *et al.*, 2008). In that study, *E. coli* O157 concentrations were greater for a longer duration in cattle fed 20% and 40% WDGS than in manure slurries from cattle fed 0% WDGS. Although the potential association between dietary distillers grains and *E. coli* O157 prevalence and/or persistence in cattle has been well described, statistically significant associations have not always been found (Jacob *et al.*, 2009). Regardless, there is no published data to suggest that distillers grains decrease the *E. coli* O157 prevalence or concentration in cattle.

The mechanism responsible for the trend of increased *E. coli* O157 when feeding distillers grains in cattle is not known. Similar to other dietary components, two general mechanisms have been proposed: (1) distillers grains alter the hindgut ecology of cattle, making a more suitable environment for *E. coli* O157, or (2) a component of distillers grains stimulates the growth of *E. coli* O157 (Jacob *et al.*, 2008a). It is not unexpected that hindgut ecology changes when cattle are fed distillers grains. Klopfenstein *et al.* (2008) described the high ruminal escape property of protein in dried distillers grain diets, which could provide more protein in the hindgut and result in increased degradation and ammonia concentration. Also, the starch content of corn has been removed in distillers grains, which allows for less secondary fermentation compared with corn-based diets. In addition, distillers grains have previously been shown to alter rumen microbial populations (Fron *et al.*, 1996). There is some evidence from *in vitro* ruminal fluid fermentations that *E. coli* O157 growth was actually stimulated compared with control fermentations; however, this was not observed in fecal fermentations, which may be expected if the site of action is the lower gut (Jacob *et al.*, 2008a). Clearly, more research is needed before this association can be explained. Physiological factors beyond those altered by feeding distillers grains likely contribute to the discrepancy between some studies, and the increasing use of distillers grains is not solely responsible for *E. coli* O157 prevalence in cattle.

Dietary Interventions

Seaweed products

There are a few reports on the ability of a commercially available brown seaweed product derived from *Ascophyllum nodosum* (Tasco-14™; Acadian, Dartmouth, Nova Scotia, Canada) to reduce *E. coli* O157 shedding in cattle. This product has been shown to improve carcass characteristics in slaughtered cattle (Braden *et al.*, 2007). Using two pens of cattle in a commercial feedlot, Braden *et al.* (2004) found that feeding 2% Tasco-14 reduced prevalence of *E. coli* O157 on hides and in fecal samples at slaughter when compared with the control group. The same product was used in a study with calves experimentally inoculated with *E. coli* O157:H7 (Bach *et al.*, 2007). Pens of inoculated calves were fed a control diet or the seaweed at different levels (10 or 20 g/kg diet) for 7 or 14 days. Over the sampling period, mean *E. coli* O157:H7 concentrations and the frequency of obtaining a positive sample were lower from animals fed Tasco-14 at 10 and 20 g/kg for 14 and 7 days, respectively, compared with animals fed the control treatment (no Tasco-14) or Tasco-14 at 20 g/kg for 14 days. The mechanism for a decrease in fecal *E. coli* O157 shedding in cattle administered Tasco-14 is not known but is hypothesized to be a direct microbial effect (Braden *et al.*, 2004). This was supported by the work of Bach *et al.* (2007), who found no changes in VFA concentrations or pH in fecal samples from the four treatments. This seaweed product may have potential as a preharvest intervention strategy.

Phenolics/essential oils

Plants commonly synthesize phenolic compounds for defense against microorganisms and predators. The antimicrobial nature of these compounds is believed to be enzyme inhibition by oxidized compounds or interactions with proteins that have not been described (Cowan, 1999). There is some evidence to suggest that phenolic compounds can be inhibitory to *E. coli* O157. Survival of *E. coli* O157 inoculated into cattle fecal samples decreased, particularly when higher concentrations (0.5%) of *trans*-cinnamic and *para*-coumaric acids were applied to the samples (Wells *et al.*, 2005). Only one of these compounds, *trans*-cinnamic acid, affected fecal pH (Wells *et al.*, 2005), so it is not known whether inhibition was pH mediated or the result of a direct microbial effect. Tannins, a more complex phenolic compound observed in hydrolyzable and condensed types (Cowan, 1999), have also been evaluated for efficacy as an inhibitory substance for *E. coli* O157. *In-vitro* incubations showed that both tannin types decreased the growth rate of *E. coli* O157 in pure culture and were bactericidal, more so with the hydrolyzable type, to *E. coli* O157 (Min *et al.*, 2007). In addition, the hydrolyzable tannin was shown to reduce fecal *E. coli* concentration in an *in vivo* experiment with cattle fed hay diets (Min *et al.*, 2007).

It has been known for some time that essential oils, which are also phenolic compounds, obtained from plants have antibacterial properties that can inhibit foodborne pathogens in pure culture (Dabbah *et al.*, 1970; Burt and Reinders, 2003; Burt, 2004). Often, these oils are more effective against Gram-positive organisms (Dabbah *et al.*, 1970; Fisher and Phillips, 2006), although application of some plant oils has been shown to reduce coliform counts in stored manure (Varel and Miller, 2001). Although the specific mechanism of action is compound dependent, the ability of oils to disrupt membranes and ion concentrations generates their antibacterial properties (Cowan, 1999; Burt, 2004). Nannapaneni *et al.* (2008) demonstrated the susceptibility of *E. coli* O157:H7 isolates to two of seven orange essential oils by agar-disk diffusion. Recently, *in vitro* ruminal fluid fermentations with varying concentrations of orange peel or dried orange pulp, which contain essential oils, were shown to decrease inoculated *E. coli* O157:H7 (Callaway *et al.*, 2008). The concentration of citrus oil reaching the lower gut, the colonization site for *E. coli* O157, is unknown. However, because the organism can also be in the rumen (Laven *et al.*, 2003) feeding orange pulp products may be a useful in-feed intervention strategy. Feeding citrus products, primarily dried pulp, to cattle as a source of energy is common in citrus-producing regions like Florida (Wing, 2003). Further work is needed to assess *in vivo* efficacy of these products; however, if proven to work they are potentially useful, particularly in diets of beef and dairy cattle in regions where the coproducts are available.

Mechanisms and Implications

It is generally accepted that most cattle shedding *E. coli* O157 do so at a concentration of $<10^3$ CFU/g feces; however, there appears to be extreme individual animal variation, with some shedding the organism at much higher levels (e.g., $>10^4$ CFU/g feces; Low *et al.*, 2005; Chase-Topping *et al.*, 2007). These animal variations, particularly when animals shed a large concentration of *E. coli* O157, likely contribute to overall group prevalence (Matthews *et al.*, 2006) but have not always been linked to diet and are not well understood. Other factors, including season, have frequently been associated with *E. coli* O157 prevalence. These factors show that the biological relationship between *E. coli* O157 and the ruminant reservoir is likely more complex than diet influences alone. However, because feed components continue to be associated with prevalence, understanding these interactions may allow us to exploit the mechanisms for potential preharvest intervention. It is difficult to assess the specific effect of different diet components on *E. coli* O157 growth and colonization *in vivo*. The true prevalence of *E. coli* O157 independent of any diet influence in cattle is not known so differences attributed to one component increasing the shedding cannot easily be distinguished from another component decreasing shedding.

The difference in prevalence observed between different diets is often attributed to changes in hindgut ecology, primarily pH and VFA concentrations, although in the case of phenolic and seaweed interventions, it may be direct microbial effects. The pH and VFA concentrations throughout the rumen and intestine are directly related to feed composition; however, studies evaluating dietary influences on *E. coli* O157 rarely report these values. Even when reported, results are not always consistent and provide only a generalized hypothesis. One reason for the inconsistencies in pH and VFA concentration data may be the inherent differences in component utilization between animals. Ørskov (1986) reports a difference in starch fermentation of 35% between two sheep fed an identical corn diet. Another reason for inconsistencies is the variability in nutrient composition between feed products including silage where the starch content is influenced by inclusion level and plant maturity (Huntington, 1997). Finally, dietary influences are sometimes reported using generic *E. coli* populations as a model for *E. coli* O157. There are inconsistencies in the response of *E. coli* O157 and more generic *E. coli* populations to pH and dietary influences. When used as a model, these results are difficult to interpret and suggest that *E. coli* may not always be equivalent for assessing an *E. coli* O157 response to diet influences (Grauke *et al.*, 2003).

One proposed mechanism for *E. coli* O157 inhibition in the hindgut is an increase in secondary starch fermentation (Fox *et al.*, 2007). Starch content can vary by grain type and processing method, but passage rate and consumption also contribute to the amount of starch initially fermented in the rumen (Huntington, 1997). Generally, a large percentage of starch (80–95%) is fermented in the rumen, and a considerable portion of the remaining starch undergoes digestion in the small intestine (Huntington, 1997). Still, starch that escapes the rumen and small intestine can undergo secondary fermentation in the large intestine, similar to ruminal fermentation, and result in changes in pH and VFA concentrations (Ørskov *et al.*, 1970). The effect of starch or glucose infused both ruminally and/or abomasally into steers was shown to lower cecal and fecal pH compared with controls; however, total anaerobic and aerobic counts were higher (Van Kessel *et al.*, 2002). There was no statistically significant difference in total *E. coli* counts. Diets with higher starch contents generally decrease the concentration of acetate while increasing propionate and butyric acid concentrations (Ørskov *et al.*, 1970). Still, more specific effects of these specific short-chain fatty acids are not well described. In pure culture, propionate was shown to reduce viability of *E. coli* O157 at 37°C (McWilliam Leitch and Stewart, 2002). Antimicrobial activity toward *E. coli* O157 in this study was actually greater for lactate, another organic acid derived from glucose. Sensitivity of *E. coli* O157 to lactate has been described elsewhere (Jordan *et al.*, 1999). In addition, Krause *et al.* (2003) showed that lactic acid bacteria showed results similar to *E. coli*, increasing in concentration in grain-fed cattle. The effect of these results, specifically as they relate to *E. coli* O157, is not known; however, lactic acid bacteria have been shown to have anti–*E. coli* O157 effects (Brashears *et al.*, 2003).

Few studies relate dietary fiber content to *E. coli* O157 shedding in cattle, and fiber content is often not measured in fecal samples. However, dietary fiber is known to alter physiology and stimulate growth of bacteria in the human colon (Cummings and Stephen, 1980). Possibly, increased fiber content stimulates increased mucus production in the hindgut. Using *in vitro* models, Fox *et al.* (2008) showed that mucus components, particularly gluconic acid, stimulated *E. coli* O157 growth. Higher fiber content was associated with *E. coli* O157 decline in inoculated fecal samples from dairy cattle fed different forage diets (Franz *et al.*, 2005). Additionally, Lema *et al.* (2002) reported that lambs inoculated with *E. coli* O157 and fed 5% dietary acid detergent fiber shed a significantly higher concentration than animals with a higher dietary acid detergent fiber percentage (10–35%). In both studies, increasing fecal pH values were seen with higher fiber content samples. Higher fiber content may be confounded by other dietary influences such as starch because of diet composition. The association between *E. coli* O157 and distillers grains, with concentrated fiber components and decreased starch content, is just one example of this complexity. Feeding high lipid content may also alter the hindgut ecosystem and affect *E. coli* O157; however, studies assessing various oils (canola and cottonseed) have generally shown no association. The lipid content in distillers grains is also concentrated, but again, these results are likely confounded by other factors (starch, fiber, etc.). Interestingly, essential oils, including those from citrus products, have some direct antimicrobial property, have shown some efficacy, and may be a useful intervention strategy.

In conclusion, to better understand effects of diet on *E. coli* O157 colonization and shedding in cattle, more specific work to confirm and identify differences beyond pH and VFA concentrations is needed. Many inconsistencies regarding dietary influences on *E. coli* O157 are reported in the literature; however, variability in nutrient composition, animal utilization, and processing methods influence these physiological conditions and make repeatable results challenging. Although difficult, work to distinguish between confounding factors such as fiber and starch may add clarity to any potential mechanism associated with increased *E. coli* O157 colonization. In addition, the organism–host relationship is likely far more complex than dietary influences alone, and the response to dietary changes of other microbial populations, possibly other foodborne pathogens, is not known. Still, if simple mechanisms can be exploited or existing or new compounds with direct anti–*E. coli* O157 activity can be developed, practical preharvest intervention strategies to reduce the economic and human health burden of this organism can be implemented.

Disclosure Statement

No competing financial interests exist.

References

Bach SJ, McAllister TA, Veira DM, Gannon VPJ, and Holley RA. Transmission and control of *Escherichia coli* O157:H7—a review. Can J Anim Sci 2002;82:475–490.

Bach SJ, Selinger LJ, Stanford K, and McAllister TA. Effect of supplementing corn- or barley-based feedlot diets with canola oil on faecal shedding of *Escherichia coli* O157:H7 by steers. J Appl Microbiol 2005a;98:464–475.

Bach SJ, Stanford K, and McAllister TA. Survival of *Escherichia coli* O157:H7 in feces from corn- and barley-fed steers. FEMS Microbiol Lett 2005b;252:25–33.

Bach SJ, Wang Y, and McAllister TA. Effect of feeding sun-dried seaweed (*Ascophyllum nodosum*) on fecal shedding of *Escherichia coli* O157:H7 by feedlot cattle and on growth performance of lambs. Anim Feed Sci Technol 2007;142:17–32.

Berg J, McAllister T, Bach S, Stilborn R, Hancock D, and LeJeune J. *Escherichia coli* O157:H7 excretion by commercial feedlot cattle fed either barley- or corn-based finishing diets. J Food Prot 2004;67:666–671.

Braden KW, Blanton JR Jr., Allen VG, Pond KR, and Miller MF. *Ascophyllum nodosum* supplementation: a preharvest intervention for reducing *Escherichia coli* O157:H7 and *Salmonella* spp. in feedlot steers. J Food Prot 2004;67:1824–1828.

Braden KW, Blanton JR Jr., Montgomery JL, van Santen E, Allen VG, and Miller MF. Tasco supplementation: effects on carcass characteristics, sensory attributes, and retail display shelf-life. J Anim Sci 2007;85:754–798.

Brashears MM, Jaroni D, and Trimble J. Isolation, selection, and characterization of lactic acid bacteria for a competitive exclusion product to reduce shedding of *Escherichia coli* O157:H7 in cattle. J Food Prot 2003;66:355–363.

Buchko SJ, Holley RA, Olson WO, Gannon VPJ, and Veira DM. The effect of different grain diets on fecal shedding of *Escherichia coli* O157:H7 by steers. J Food Prot 2000;63:1467–1474.

Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int J Food Microbiol 2004;94: 223–253.

Burt SA and Reinders RD. Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. Lett Appl Microbiol 2003;36:162–167.

Callaway TR, Anderson RC, Edrington TS, Elder RO, Genovese KJ, Bischoff KM, Poole TL, Jung YS, Harvey RB, and Nisbet DJ. Preslaughter intervention strategies to reduce food-borne pathogens in food animals. J Anim Sci 2003a;81(E. Suppl 2):E17–E23.

Callaway TR, Elder RO, Keen JE, Anderson RC, and Nisbet DJ. Forage feeding to reduce preharvest *Escherichia coli* populations in cattle, a review. J Dairy Sci 2003b;86:852–860.

Callaway TR, Carroll JA, Arthington JD, Pratt C, Edrington TS, Anderson RC, Galyean ML, Ricke SC, Crandall P, and Nisbet DJ. Citrus products decrease growth of *E. coli* O157:H7 and *Salmonella* Typhimurium in pure culture and in fermentation with mixed ruminal microorganisms *in vitro*. Foodborne Pathog Dis 2008;5:1–7.

Chase-Topping ME, McKendrick IJ, Pearce MC, MacDonald P, Matthews L, Halliday J, Allison L, Fenlon D, Low JC, Gunn G, and Woolhouse MEJ. Risk factors for the presence of high-level shedders of *Escherichia coli* O157 on Scottish farms. J Clin Microbiol 2007;45:1594–1603.

Chase-Topping ME, Gally D, Low C, Matthews L, and Woolhouse MEJ. Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. Nat Rev Microbiol 2008;6:904–912.

Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999;12:564–582.

Cummings JH and Stephen AM. The role of dietary fibre in the human colon. CMA J 1980;123:1109–1114.

Dabbah R, Edwards VM, and Moats WA. Antimicrobial action of some citrus fruit oils on selected food-borne bacteria. Appl Microbiol 1970;19:27–31.

Dargatz DA, Wells SJ, Thomas LA, Hancock DD, and Garber LP. Factors associated with the presence of *Escherichia coli* O157 in feces of feedlot cattle. J Food Prot 1997;60:466–470.

Depenbusch BE, Nagaraja TG, Sargeant JM, Drouillard JS, Loe ER, and Corrigan ME. Influence of processed grains on fecal pH, starch concentration, and shedding of *Escherichia coli* O157 in feedlot cattle. J Anim Sci 2008;86:632–639.

Dewell GA, Ransom JR, Dewell RD, McCurdy K, Gardner IA, Hill AE, Sofos JN, Belk KE, Smith GC, and Salman MD. Prevalence of and risk factors for *Escherichia coli* O157 in market-ready beef cattle from 12 U.S. feedlots. Foodborne Pathog Dis 2005;2:70–76.

Diez-Gonzalez F, Callaway TR, Kizoulis MG, and Russell JB. Grain feeding and the dissemination of acid-resistant *Escherichia coli* from cattle. Science 1998;281:1666–1668.

Fegan N, Vanderlinde P, Higgs G, and Desmarchelier P. The prevalence and concentration of *Escherichia coli* O157 in faeces of cattle from different production systems at slaughter. J Appl Microbiol 2004;97:362–370.

Fisher K and Phillips CA. The effect of lemon, orange, and bergamot essential oils and their components on the survival of *Campylobacter jejuni*, *Escherichia coli* O157, *Listeria monocytogenes*, *Bacillus cereus* and *Staphylococcus aureus in vitro* and in food systems. J Appl Microbiol 2006;101:1232–1240.

Fox JT, Depenbusch BE, Drouillard JS, and Nagaraja TG. Dry-rolled or steam-flaked grain-based diets and fecal shedding of *Escherichia coli* O157 in feedlot cattle. J Anim Sci 2007;85:1207–1212.

Fox JT, Drouillard JS, Shi X, and Nagaraja TG. Effects of mucin and its carbohydrate constituents on *Escherichia coli* O157 growth in batch culture fermentations with ruminal or fecal microbial inoculums. J Anim Sci 2009;87:1304–1313.

Franz E, van Diepeningen AD, de Vos OJ, and van Bruggen AHC. Effects of cattle feeding regimen and soil management type on the fate of *Escherichia coli* O157:H7 and *Salmonella enteric* serovar Typhimurium in manure, manure-amended soil, and lettuce. Appl Environ Microbiol 2005;71:6165–6174.

Fron M, Madeira H, Richards C, and Morrison M. The impact of feeding condensed distillers byproducts on rumen microbiology and metabolism. Anim Feed Sci Technol 1996;61:235–245.

Fu CJ, Porter JH, Felton EED, Lehmkuhler JW, and Kerley MS. Pre-harvest factors influencing the acid resistance of *Escherichia coli* and *E. coli* O157:H7. J Anim Sci 2003;81:1080–1087.

Galbraith H and Miller TB. Effect of metal cations and pH on the antibacterial activity and uptake of long chain fatty acids. J Appl Bacteriol 1973;36:635–646.

Gannon VPJ, Graham TA, King R, Michel P, Read S, Ziebell K, and Johnson RP. *Escherichia coli* O157:H7 infection in cows and calves in a beef cattle herd in Alberta, Canada. Epidemiol Infect 2002;129:163–172.

Garber LP, Wells SJ, Hancock DD, Doyle MP, Tuttle J, Shere JA, and Zhao T. Risk factors for fecal shedding of *Escherichia coli* O157:H7 in dairy calves. J Am Vet Med Assoc 1995;207:46–49.

Gilbert RA, Tomkins N, Padmanabha J, Gough JM, Krause DO, and McSweeney CS. Effect of finishing diets on *Escherichia coli* populations and prevalence of enterohaemorrhagic *E. coli* virulence genes in cattle faeces. J Appl Microbiol 2005;99:885–894.

Grauke LJ, Wynia SA, Sheng HQ, Yoon JW, Williams CJ, Hunt CW, and Hovde CJ. Acid resistance of *Escherichia coli* O157:H7 from the gastrointestinal tract of cattle fed hay or grain. Vet Microbiol 2003;95:211–225.

Hancock DD, Besser TE, Kinsel ML, Tarr PI, Rice DH, and Paros MG. The prevalence of *Escherichia coli* O157:H7 in dairy and beef cattle in Washington State. Epidemiol Infect 1994;113: 119–207.

Herriott DE, Hancock DD, Ebel ED, Carpenter LV, Rice DH, and Besser TE. Association of herd management factors with colonization of dairy cattle by shiga toxin-positive *Escherichia coli* O157. J Food Prot 1998;61:802–807.

Hovde CJ, Austin PR, Cloud KA, Williams CJ, and Hunt CW. Effect of cattle diet on *Escherichia coli* O157:H7 acid resistance. Appl Environ Microbiol 1999;65:3233–3235.

Huntington GB. Starch utilization by ruminants: from basics to the bunk. J Anim Sci 1997;75:852–867.

Jacob ME, Fox JT, Drouillard JS, Renter DG, and Nagaraja TG. Effects of dried distillers' grain on fecal prevalence and growth of *Escherichia coli* O157 in batch culture fermentations from cattle. Appl Environ Microbiol 2008a;74:38–43.

Jacob ME, Parsons GL, Shelor MK, Fox JT, Drouillard JS, Thomson DU, Renter DG, and Nagaraja TG. Feeding supplemental dried distiller's grains increases faecal shedding of *Escherichia coli* O157 in experimentally inoculated calves. Zoonoses Public Health 2008b;55:125–132.

Jacob ME, Fox JT, Drouillard JS, Renter DG, and Nagaraja TG. Evaluation of feeding dried distiller's grains with solubles and dry-rolled corn on the fecal prevalence of *Escherichia coli* O157:H7 and *Salmonella* spp. in cattle. Foodborne Pathog Dis 2009;6:145–153.

Jordan SL, Glover J, Malcolm L, Thomson-Carter FM, Booth IR, and Park SF. Augmentation of killing of *Escherichia coli* O157 by combinations of lactate, ethanol, and low-pH conditions. Appl Environ Microbiol 1999;65:1308–1311.

Klopfenstein TJ, Erickson GE, and Bremer VR. Board-invited review: use of distillers by-products in the beef cattle feeding industry. J Anim Sci 2008;86:1223–1231.

Krause DO, Smith WJM, Conlan LL, Gough JM, Williamson MA, and McSweeney CS. Diet influences the ecology of lactic acid bacteria and *Escherichia coli* along the digestive tract of cattle: neural networks and 16S rDNA. Microbiology 2003; 149:57–65.

Kudva IT, Hatfield PG, and Hovde CJ. Effect of diet on the shedding of *Escherichia coli* O157:H7 in a sheep model. Appl Environ Microbiol 1995;61:1363–1370.

Kudva IT, Hunt CW, Williams CJ, Nance UM, and Hovde CJ. Evaluation of dietary influences on *Escherichia coli* O157:H7 shedding by sheep. Appl Environ Microbiol 1997;63:3878–3886.

Laven RA, Ashmore A, and Stewart CS. *Escherichia coli* in the rumen and colon of slaughter cattle, with particular reference to *E. coli* O157. Vet J 2003;165:78–83.

LeJeune JT and Wetzel AN. Preharvest control of *Escherichia coli* O157 in cattle. J Anim Sci 2007;85(13 Suppl):E73–E80.

Lema M, Williams L, Walker L, and Rao DR. Effect of dietary fiber on *E. coli* O157:H7 shedding in lambs, 2002. Small Ruminant Res 2002;43:249–255.

Loneragan GH and Brashears MM. Pre-harvest interventions to reduce carriage of *E. coli* O157 in harvest-ready feedlot cattle. Meat Sci 2005;71:72–78.

Low JC, McKendrick IJ, McKechnie C, Fenlon D, Naylor SW, Currie C, Smith DGE, Allison L, and Gally DG. Rectal carriage of enterohemorrhagic *Escherichia coli* O157 in slaughtered cattle. Appl Environ Microbiol 2005;71:93–97.

Matthews L, Low JC, Gally DL, Pearce MC, Mellor DJ, Heesterbeek JAP, Chase-Topping M, Naylor SW, Shaw DJ, Reid SWJ, Gunn GJ, and Woolhouse MEJ. Heterogeneous shedding of *Escherichia coli* O157 in cattle and its implications for control. Proc Natl Acad Sci 2006;103:547–552.

McWilliam Leitch EC and Stewart CS. Susceptibility of *Escherichia coli* O157 and non-O157 isolates to lactate. Lett Appl Microbiol 2002;35:176–180.

Min BR, Pinchak WE, Anderson RC, and Callaway TR. Effect of tannins on the *in vitro* growth of *Escherichia coli* O157:H7 and *in vivo* growth of generic *E. coli* excreted from steers. J Food Prot 2007;70:543–550.

Nannapaneni R, Muthaiyan A, Crandall PG, Johnson MG, O'Bryan CA, Chalova VI, Callaway TR, Carroll JA, Arthington JD, Nisbet DJ, and Ricke SC. Antimicrobial activity of commercial citrus-based natural extracts against *Escherichia coli* O157:H7 isolates and mutant strains. Foodborne Pathog Dis 2008;5:695–699.

Naylor SW, Low JC, Besser TE, Mahajan A, Gunn GJ, Pearce MC, McKendrick IJ, Smith DGE, and Gally DL. Lymphoid follicle-dense mucosa at the terminal rectum is the principal site of colonization of enterohemorrhagic *Escherichia coli* O157:H7 in the bovine host. Infect Immun 2003;71:1505–1512.

Ørskov ER. Starch digestion and utilization in ruminants. J Anim Sci 1986;63:1624–1633.

Ørskov ER, Fraser C, Mason VC, and Mann SO. Influence of starch digestion in the large intestine of sheep on caecal fermentation, caecal microflora and faecal nitrogen excretion. Br J Nutr 1970;24:671–682.

Peterson RE, Klopfenstein TJ, Moxley RA, Erickson GE, Hinkley S, Rogan D, and Smith DR. Efficacy of dose regimen and observation of herd immunity from a vaccine against *Escherichia coli* O157:H7 for feedlot cattle. J Food Prot 2007;70:2561–2567.

Rangel JM, Sparling PH, Crowe C, Griffin PM, and Swerdlow DL. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982–2002. Emerg Infect Dis 2005;11:603–609.

Renter DG, Smith DR, King R, Stilborn R, Berg J, Berezowski J, and McFall M. Detection and determinants of *Escherichia coli* O157:H7 in Alberta feedlot pens immediately prior to slaughter. Can J Vet Res 2008;72:217–227.

Russell JB, Diez-Gonzalez F, and Jarvis DN. Invited review: effects of diet shifts on *Escherichia coli* in cattle. J Dairy Sci 2000; 83:863–873.

Sargeant JM, Sanderson MW, Smith RA, Griffin DD. *Escherichia coli* O157 in feedlot cattle feces and water in four major feeder-cattle states in the USA. Prev Vet Med 2003;61:127–135.

Shin R, Suzuki M, and Morishita Y. Influence of intestinal anaerobes and organic acids on the growth of enterohaemorrhagic *Escherichia coli* O157:H7. J Med Microbiol 2002;51:201–206.

Synge BA, Chase-Topping ME, Hopkins GF, McKendrick IJ, Thomson-Carter F, Gray D, Rusbridge SM, Munro FI, Foster G, and Gunn GJ. Factors influencing the shedding of verocytotoxin-producing *Escherichia coli* O157 by beef suckler cows. Epidemiol Infect 2003;130:301–312.

Theurer CB. Grain processing effects on starch utilization by ruminants. J Anim Sci 1986;63:1649–1662.

Tkalcic S, Brown CA, Harmon BG, Jain AV, Mueller EPO, Parks A, Jacobsen KL, Martin SA, Zhao T, and Doyle MP. Effects of diet on rumen proliferation and fecal shedding of *Escherichia coli* O157:H7 in calves. J Food Prot 2000;63:1630–1636.

Van Baale MJ, Sargeant JM, Gnad DP, DeBey BM, Lechtenberg KF, and Nagaraja TG. Effect of forage or grain diets with or without monensin on ruminal persistence and fecal *Escherichia coli* O157:H7 in cattle. Appl Environ Microbiol 2004;70:5336–5342.

Van Kessel JS, Nedoluha PC, Williams-Campbell A, Baldwin RL VI, and McLeod KR. Effects of ruminal and postruminal infusion of starch hydrolysate or glucose on the microbial ecology of the gastrointestinal tract in growing steers. J Anim Sci 2002;80:3027–3034.

Varel VH and Miller DN. Plant-derived oils reduce pathogens and gaseous emissions from stored cattle waste. Appl Environ Microbiol 2001;67:1366–1370.

Varel VH, Wells JE, Berry ED, Spiehs MJ, Miller DN, Ferrell CL, Shackelford SD, and Koohmaraie M. Odorant production and persistence of *Escherichia coli* in manure slurries from cattle fed zero, twenty, forty, or sixty percent wet distillers grains with solubles. J Anim Sci 2008;86:3617–3627.

Wells JE, Berry ED, and Varel VH. Effects of common phenolic acids on *Escherichia coli* O157:H7 viability in feces. Appl Environ Microbiol 2005;71:7974–7979.

Wing JM. Citrus feedstuffs for dairy cattle. Univ Fla IFAS Extension 2003 (BUI 829).

Address correspondence to:
T.G. Nagaraja, Ph.D.
Department of Diagnostic Medicine and Pathobiology
Kansas State University
305 Coles Hall
1800 Denison Ave.
Manhattan, KS 66503-8663

E-mail: tnagaraj@vet.k-state.edu